Outbreak of sheeppox in farmed sheep in Kyrgystan: Histological, eletron microscopical and molecular characterization

Aldaiarov, N ; Stahel, A ; Nufer, L ; Jumakanova, Z ; Tulobaev, A ; Ruetten, M

Abstract: INTRODUCTION On a farm in the Kyrgyz Republic, several dead sheep were found without any history of illness. The sheep showed several ulcerations on lips and bare-skinned areas. At necropsy the lungs showed multiple firm nodules, which were defined as pox nodules histologically. In the rumen hyperkeratotic plaques were visible. With electron microscopy pox viral particles were detected and confirmed with q PCR as Capripoxviruses. Although all members of the Capripoxvirus genus are eradicated in western countries, this study should remind us of the classical lesions observed in poxvirus infections.

DOI: https://doi.org/10.17236/sat00076

Outbreak of sheeppox in farmed sheep in Kyrgystan: Histological, eletron microscopical and molecular characterization

N. Aldaiarov[1], A. Stahel[2], L. Nufer[3], Z. Jumakanova[4], A. Tulobaev[4], M. Ruetten[5]

[1] Biology Department of Natural Sciences Faculty, Kyrgyz-Turkish Manas University, Bishkek, Kyrgystan, [2] Institute of Virology and [3] Institute of Veterinary Pathology, Vetsuisse Faculty, University Zürich, Switzerland, [4] Department of Veterinary Pathology, Veterinary Faculty, Kyrgyz-Turkish Manas University, Bishkek, Kyrgystan, [5] PathoVet AG, Tagelswangen, Switzerland

Summary

On a farm in the Kyrgyz Republic, several dead sheep were found without any history of illness. The sheep showed several ulcerations on lips and bare-skinned areas. At necropsy the lungs showed multiple firm nodules, which were defined as pox nodules histologically. In the rumen hyperkeratotic plaques were visible. With electron microscopy pox viral particles were detected and confirmed with q PCR as Capripoxviruses. Although all members of the *Capripoxvirus* genus are eradicated in western countries, this study should remind us of the classical lesions observed in poxvirus infections.

Keywords: *Capripoxvirus*, sheep, Kyrgyz Republic, histology, electron microscopy

Ausbruch von Schafpocken in Kirgistan: eine detaillierte histologische, elektronenmikroskopische und molekulare Charakterisierung

Auf einem Bauernhof in Kirgistan wurden mehrere tote Schafe ohne vorherige Erkrankung aufgefunden. Die Schafe zeigten an Lippen und an wenig behaarten Stellen des Körpers kleine Ulzerationen. In der Lunge konnten derbe Knoten ertastet werden, die mittels Histologie als "Poxknoten" diagnostiziert wurden. Im Pansen befanden sich hyperkeratotische "Plaques". Bei der elektonenmikroskopischen Untersuchung wurden im Zytoplasma der Pneumozyten typische Poxviruspartikel dargestellt und mittels q PCR als Capripoxviren identifiziert. Obwohl Infektionen der Gattung *Caprioxvirus* in westlichen Ländern nicht mehr vorkommen, soll diese Arbeit die Läsionen bei einer Pockeninfektion wieder in Erinnerung zu rufen.

Schlüsselwörter: *Capropoxvirus*, Schafe, Kyrgistan, Histologie, Elektronenmikroskopie

DOI 10.17236/sat00076

Received: 12.10.2015
Accepted: 04.11.2015

Introduction and case history

On a farm in the region of Naryn and Talas in the Kyrgyz Republic, several dead sheep were found without any history of illness. The animals were not vaccinated or treated against any disease. Seven animals were sent to the Natural Science Faculty's Biology Department at the Kyrgyz-Turkish Manas University in Bishkek for further investigation. At necropsy the sheep were thin to kachectic. The animals presented with round to oval, slightly raised, well demarcated swellings or vesicles of up to 2 cm in diameter (Fig. 1C and 1D). Some vesicles were ruptured, leaving red ulcerations behind on the short-haired to bare-skinned areas of the body and on the mucous membranes around the eyes, nose, muzzle, anus, and the genitals (Fig. 1A). Some ulcerations were surrounded by a red rim, others were surrounded by granulation tissue. The animals had often a purulent discharge from the eyes and nostrils (Fig. 1A and 1B). The lungs showed multifocal, randomly distributed, grayish necroses in the caudal lung lobes, which were clearly demarcated. The pleura overlaying these nodules was thickened and firm (Fig. 1E). The ruminal mucosa showed multifocal hyperplastic and hyperkeratotic, grayish, round, slightly upraised plaques (Fig. 1F). The lymph nodes were small and flabby.

Histology

Histologically, the epidermis was multifocally ulcerated and the defects often covered with crusts consisting of fibrin mixed with cellular debris and dirt. The necroses extended into the superficial dermis often involving hair follicles and adnexa. The remaining epidermis formed small vesicles that were filled with inflammatory exudate containing free floating, acantholytic epithelial cells. Eventually, some vesicles were ruptured and the underlying tissue was severely infiltrated by numerous degenerating neutrophils and fewer macrophages. The remaining epidermis was thickened by acanthosis covered by marked para- and orthokeratosis (Fig. 2A). The keratinocytes of the basal to mid cell layers were often swollen by hydropic influx and often hypergranulated. The nuclei were often round and enlarged with chromatin margination. The cells contained round to oval eosinophilic, intracytoplasmatic inclusion bodies of up to 2 µm in diameter (Fig. 2B). The small blood vessels in the dermis often showed a degenerating, necrotizing endothelium with fibrin depositions in the media of the vessels. The pleura covering the firm nodules of the lung parenchyma was thickened by fibrosis with mild mixed inflammation. Some of the proliferated fibroblasts showed the ovoid eosinophilic intracytoplasmatic viral inclusion bodies and were therefore called "Borrel or sheep pox cells" (Fig. 2C). The firm, small "pox nodules" were randomly distributed in the lung parenchyma and consisted of hyperplastic respiratory epithelium in smaller airways or hyperplastic type II pneumocytes lining the alveolar spaces. The epithelium exhibited severe proliferation of up to 4 cell layers thick, causing almost complete occlusion of small bronchiolar airways leaving only a small lumen for the passage of air. The superficial respiratory epithelial cells often showed loss of cilia and metaplasia to squamous epithelium. Occasionally, the proliferated pneumocytes showed the same eosinophilic intracytoplasmic inclusions (Fig. 2D) as seen in the skin. The alveolar spaces were filled with protein-rich fluid and an increased number of alveolar macrophages which themselves occasionally contained intracytoplasmic inclusions.

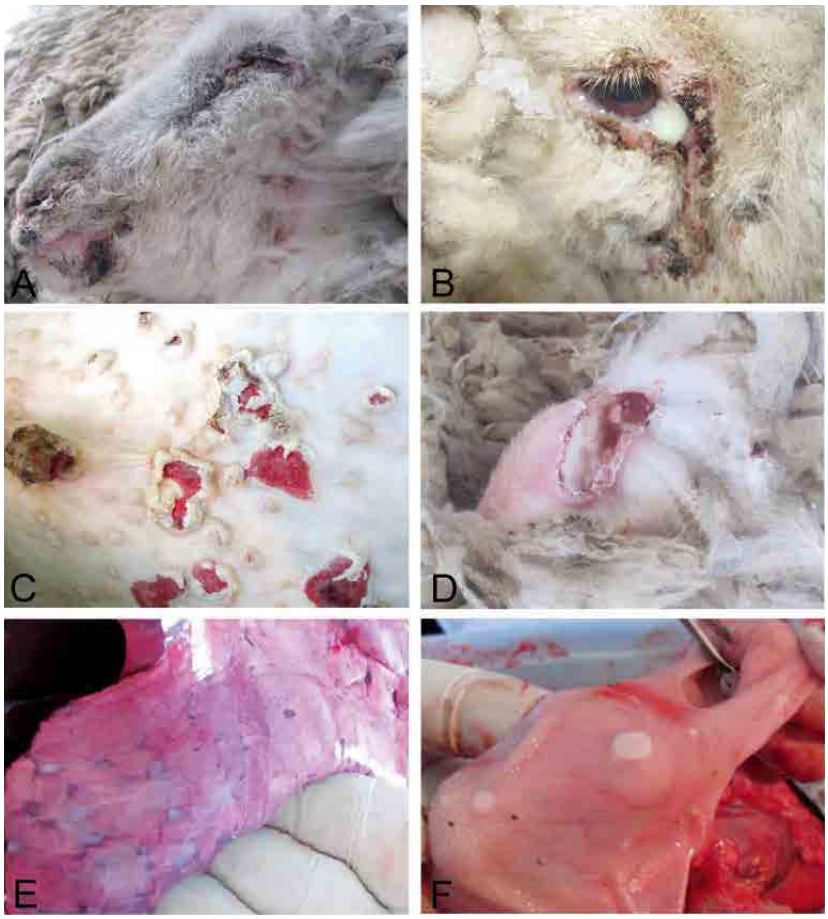

Figure 1: Macroscopical images of affected animals showing upraised erosions and reddened skin on muzzle (A), perianal (D) and abdominal wall (C). Purulent ocular discharge is visible (B). The surface of the lungs (E) and rumen (F) show multiple, firm, beige, round plaques.

Electron microscopy

Electron micrographs were taken of hyperplastic pneumocytes which under the light microscope revealed intracytoplasmic inclusion bodies, intracytoplasmal oedema (Fig. 2E), dilation and lysis of mitochondria. Several large (up to 350 nm), brick shaped, enveloped virions with the characteristic pox virus nucleocapsid (Fig. 2F) were detected. The nuclei showed chromatin margination and central pallor with electron-lucent, fibril-filled karyolytic regions (Fig. 2E).

Molecular analysis

In order to confirm the microscopical findings, DNA was extracted from 30 µm thin paraffin sections using the QIAamp DNA Mini Kit (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer's instructions. Detection of Capripoxvirus by PCR assay utilised genus specific primers and probe (Balinsky *et al.*, 2008) and was performed on a 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham MA, USA) with standard ramping conditions. The sample analysed reacted positively for Capripoxvirus.

Diagnosis

The morphological diagnosis was a multifocal, ulcerative and proliferative dermatitis with intracytoplasmatic, epidermal viral inclusions and a multifocal, interstitial

and proliferative pneumonia with intracytoplasmatic viral inclusions in pneumocytes and macrophages. The macroscopical and histological lesions as seen in the skin and lungs are typical for *Poxviridae* infections in all species. Similar lesions were observed in the rumen and additionally the lymphatic tissue was severely depleted as it is described by the OIE (OIE, 2009). These morphological diagnoses could be confirmed by electron microscopy showing typical pox virions in the cytoplasm of pneumocytes.

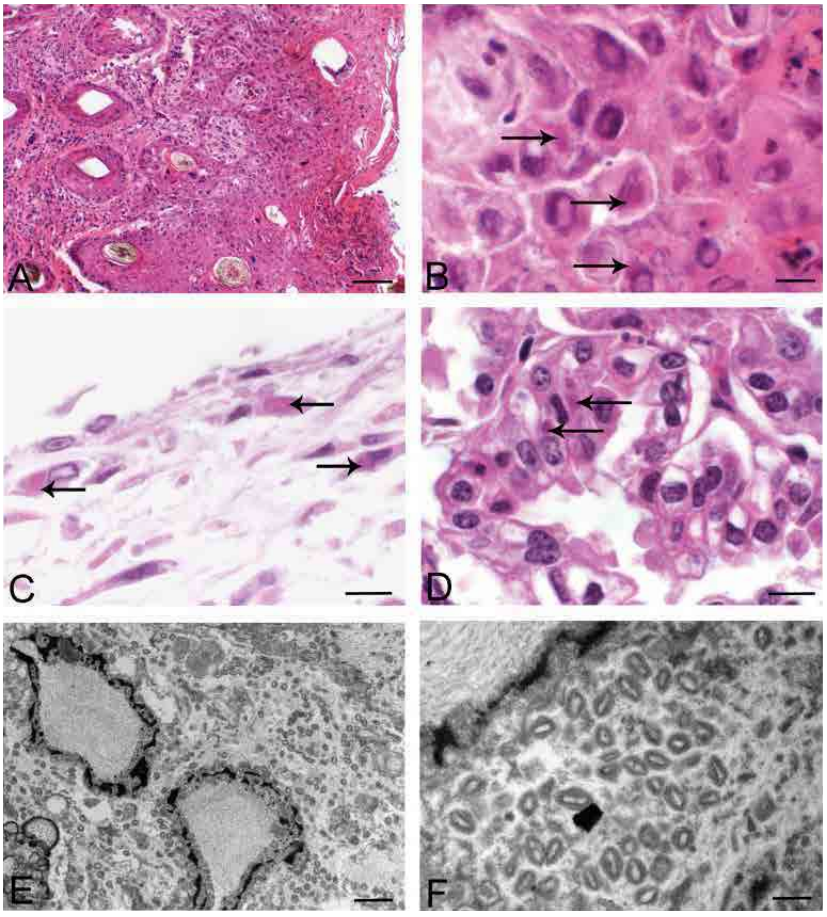

Figure 2: Histological images: A: Affected skin showing the classical proliferative lesions of the epidermis. H&E staining, bar = 100 µm. B: Higher magnification of keratinocytes showing the basophilic, intracytoplasmatic viral inclusions (arrows) and chromatin margination. H&E staining, bar = 10 µm. C: Thickening of pleura by proliferation of fibroblasts containing intracytoplasmatic viral inclusions "Borrel cells" (arrows). H&E staining, bar = 10 µm. D: High magnification of pneumocytes type II with intracytoplasmatic, eosinophilic viral inclusions (arrows). H&E staining, bar = 10 µm. E: Electron micrograph of an epithelial cell showing degeneration, intracytoplasmic oedema, chromatin margination, central pallor with electron-lucent, fibril-filled karyolytic regions and intracytoplasmic virions. Bar = 5 µm. F: Higher magnification electron micrograph of the epithelial cell shown in E, typical pox virions with "brick stone" appearance. Bar = 2 µm.

Disscusion

The Kyrgyz Republic is a mountainous, landlocked country of 198'500 km^2 bordering Kazakhstan, Uzbekistan, Tajikistan and China. The Kyrgyz Republic is classified as a low income country with an average gross domestic product (GDP) per capita of $US 864, compared to western countries ranging between $US 22'130 to 111'716 (World Bank Poverty Report, 2007). The majority (65%) of the population is rural and 31% of the total workforce comes from the agricultural sector. Agriculture and livestock remain the backbone of the economy, providing substantial employment, playing critical roles in both price stability and as a leading source of export earnings. A particularly serious problem is the consumption of meat from diseased animals, which has a great impact on public health. Brucellosis, echinococcosis, foot-and-mouth disease, peste des petits ruminants, sheep and goatpox as well as parasites are widespread throughout the country (Japaraliev, 2011), severely impacting on productivity and profitability, and posing a considerable zoonotic risk. Official statistical data on morbidity and mortality of the sheep and goat population due to poxvirus infections are lacking. However, farmers report approximately 10–30% of all young and mature animals die from this disease. The health of animals has deteriorated with the reduction of veterinary services following Soviet times. There are attempts to strengthen the services, primarily through support of private veterinarians and in particular by building up a capable public veterinary health service. Sheep and goat pox, are amongst the most highly contagious and reportable diseases of small ruminants (Babiuk *et al.*, 2008; Japaraliev, 2011; Kitching and Carn, 2010). The disease is widespread and has been registered in 57 countries distributed over Africa, Asia, and the European continent (OIE, 2008, Das *et al.*, 2012). The *Capripoxvirus* genus of the *Poxviridae* family and subfamily chordopoxvirinae comprises three viruses: sheeppox (SPPV), goatpox (GTPV) and lumpy skin disease (LSDV). They are double stranded, enveloped DNA viruses and share a similar nucleotide sequence with up to 99% identity (Le Goff *et al.*, 2009; Tulman *et al.*, 2002). LSDV causes disease in cattle and occurs in Egypt, Israel, and in African countries adjacent to the Sahara (Babiuk *et al.*, 2008; Bhanuprakash *et al.*, 2006; Diallo *et al.* 2007). Although SPPV and GTPV are considered highly host specific, Kenyan and Yemen isolates are reported to be equally infective for goats and sheep (Rao and Bandyopadhyay, 2000). All ages or breeds of sheep and goats can be affected and kids and lambs are generally more susceptible than adults. The lesions in the skin are characterized by typical proliferative epidermal changes and inclusion bodies within epithelial cells; the same lesions are seen in internal organs such as lungs and rumen. Depletion of the lymphatic system is common (Beytut *et al.*, 2010). Although SPPV and GTPV are eradicated in western and industrial countries, we think it is wise to regularly remind ourselves of the classical lesions of malignant poxvirus infections in or-

der to react immediately, should an outbreak occur. The lesions differ from the site of infection and distribution compared to the more benign *Parapoxvirus* infections in goats and sheep, where internal organs are seldom affected. However, molecular testing should be considered in unclear cases.

Acknowledgements

The authors thank Prof Lloyd Vaughan for correcting the English and for critical comments on the manuscript. All authors declared to have received no funding for this short communication.

References

Animal disease data of OIE, 2008

Babiuk, S., Bowden, T. R., Boyle, D. B., Wallace, D. B., Kitching, R. P.: Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. Transbound. Emerg. Dis. 2008, 5: 263–272.

Balinsky, C. A., Delhon, G., Smoliga, G., Prarat, M., French, R. A., Geary S. J., Rock D. L., Rodriguez L. L.: Rapid preclinical detection of sheeppox virus by a real-time PCR assay. J. Clin. Microbiol. 2008, 46: 438–442.

Beytut, E.: Sheep pox virus induces proliferation of type II pneumocytes in the lungs. J. Comp. Path. 2010, 143: 132–141.

Bhanuprakash, V., Indrani, B. K., Hosamani, M., Singh, R. K.: The current status of sheep pox disease. Comp. Immunol. Microbiol. Infect. Dis. 2006, 29: 27–60.

Das, A., Babiuk, S., McIntosh, M. T.: Development of a loop-mediated isothermal amplification assay for rapid detection of capripoxviruses. J. Clin. Microbiol. 2012, 50: 1613–1620.

Diallo, A., Viljoen, G. J.: Genus capripoxvirus. In: *Poxviruses*. Hrsg.Mercer, A.A., Schmidt, A., Weber, O., Birkhauser Verlag, Basel, Switzerland. 2007.

Japaraliev, N. T.: Development of modern methods of diagnosis and specific prophylaxis of sheeppox and goatpox. DVM thesis, Manas University of Bishkek. 2011.

Kitching, R. P., Carn, V.: Sheep pox and Goat pox. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals OIE Paris. 2010.

Le Goff, C., Lamien, C. E., Fakhfakh, E., Chadeyras, A., Aba-Adulugba, E., Libeau, G., Tupurainen, E., Wallace, D. B., Adam, T., Silber, R., Gulyaz, V., Madani, H., Caufour, P., Hammami, S., Diallo, A., Albina, E.: Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination. J. Gen. Virol. 2009, 90: 1967–1977.

Rao, T. V. S. and Bandyopadhyay, S. K.: A comprehensive review of goat pox and sheep pox and their diagnosis. Animal health research reviews. 2000, 1: 127–136.

Report of the 10-th Conference of the OIE Regional Commission for the Middle-East, Doha, Qatar, October, 2009.

Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Sur, J.H., Sandybaev, N. T., Kerembekova, U. Z., Zaitec, V. L., Kutish, G. F., Rock, D. L.: The genomes of sheeppox and goatpox viruses. J. Virol. 2002, 7: 6054–6061.

World Bank Poverty Report , 2007.

Corresponding author

Maja Ruetten
PathoVet AG
CH-8317 Tagelswangen
Tel. +41 (0)52 208 99 20
E-Mail: maja.ruetten@pathovet.ch